Two potential sexing techniques for the Eastern Bristlebird *Dasyornis brachypterus*

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ABSTRACT

Over fifty percent of bird species are sexually monomorphic and the Eastern Bristlebird *Dasyornis brachypterus* was previously considered to be part of this majority. To test this suggestion, morphological characteristics were measured on live and preserved *D. brachypterus*, with sex determined genetically for 46 live individuals using a common molecular technique. Males were significantly heavier, had larger heads, longer wings and longer tails than females. Univariate sexing criteria were developed based on the differences between males and females in two of these measures, weight and head-bill length and these measures were used to sex fifteen additional birds for which sex had been determined genetically. A discriminant function was also derived from the two characters. When the discriminant function was used in conjunction with the sexing criteria, 80 % of results were consistent with molecular results, 7 % disagreed and 13 % were inconclusive. I speculate that this inaccuracy was due to juvenile males and the time of year of trapping. However, the technique can be used to sex an individual in the hand with 80% accuracy, and can therefore provide a relatively quick and inexpensive method to investigate the sex of *D. brachypterus* in the field; which has benefits for the active conservation of this endangered bird.

Key words: Eastern Bristlebird, sexing technique, discriminant function analysis

Introduction

Over fifty percent of bird species worldwide are thought to be sexually monomorphic (Griffiths *et al.* 1998), meaning that there are no discernable differences in the physical appearances of males and females. This similarity between the sexes can often lead to difficulties in undertaking ecological research on these species. The knowledge of sex ratios is particularly important in the conservation of small populations (Double and Olsen 1997; Lens *et al.* 1998) as it provides insights into their population dynamics and can inform management. To determine the sex of an individual in sexually monomorphic species three options are generally available: (i) conduct intensive behavioural studies, (ii) undertake a laparoscopy on individuals to look for testes or ovaries, or (iii) take a DNA sample and use molecular sexing techniques, once genetic markers for sex have been established for the species.

The ability to sex an individual quickly and cheaply in the hand is vital in translocation or captive breeding programs, especially when only small numbers of birds can be captured and it is essential that both males and females are included in the sample. In such situations, behavioural studies or laparoscopy may not be appropriate because of the time required or potential risks to the individuals respectively. Molecular genetic sexing techniques are extremely valuable because they pose little risk to individuals and are practical in most applications. The drawbacks to molecular sexing techniques are the time lag between sample collection and analysis and, in some circumstances, the cost. What is needed in conservation programs is a quick and cheap method of distinguishing sex that is applicable in the field.

Many of Australia's birds are of conservation concern and some of these species show little or no sexual dimorphism. Examples include the Ground Parrot *Pezoporus wallicus* and Eastern Bristlebird *Dasyornis brachypterus* (Higgins 1999; Garnett and Crowley 2000; Higgins and Peter 2002). *Dasyornis brachypterus* is listed as endangered under Australia's *Environment Protection and Biodiversity Conservation Act 1999*. It has been considered a sexually monomorphic species (Simpson and Day 1996; DEC 2004), although Chaffer (1954) suggested that, while both sexes look superficially similar, the females may be smaller than the males. Higgins and Peter (2002) have highlighted variation between the sexes but suggest a formal study is needed to investigate these differences. *D. brachypterus* is the focus of continuing conservation efforts that will be aided through an increased understanding of the species population dynamics.

This study investigates sexual dimorphism in *D. brachypterus* by investigating the relationship between morphological features and sex in individuals and specimens of *D. brachypterus* collected over the last 141 years. It develops and evaluates two types of criteria for determining sex in the field, a set of morphological criteria and a discriminant function analysis based on morphological characters.

Methods

Data Collection

Morphometrics were collated on 17 museum specimens of *D. brachypterus* collected from 1864 to 1993 and deposited with the Australian Museum (Sydney) and the Australian National Wildlife Collection (Commonwealth Science

and Industrial Research Organisation, Canberra). The sex of these birds was provided on the specimens, although the method of sexing was not provided. It is acknowledged that museum specimens may be subject to some shrinkage. Not all data was available for all specimens and only wing and tail length was collated from museum specimens that were measured after preservation (n = 13). There was no statistical difference between museum specimens and the individuals caught during this study in these measurements and so the data from museum specimens were pooled with data from live *D. brachypterus* to increase the small sample size. At Jervis Bay, NSW in Autumn 2003 and 2004, 31 *D. brachypterus* were caught using mist nets and call playback and the following morphological characters were measured: weight, flattened straightened wing length, tail length, tarsus length, head-bill length and culmen length (Rogers 1989). Three pin feathers were removed from each bird for DNA analysis, performed at the Museum of Victoria (Dr Janette Norman pers. comm.). A common DNA molecular sexing technique exploiting birds heterogameticity (i.e. male has ZZ sex chromosomes and female ZW) was used to determine the sex of each individual. This technique utilises polymerase chain reaction (PCR) to amplify two chromo-helicase-DNA-binding genes located on the sex chromosomes of most birds, expressed using gel electrophoresis (see Griffiths *et al.* 1998; Bermudez-Humaran *et al.* 2002 for details).

Sexing Criteria

Student *t*-tests were used to determine differences between males and females in individual morphological measurements. Those measures that revealed statistically significant differences between males and females were considered to be suitable for sexing. As an independent test of the measures that were finally selected, another 15 *D. brachypterus* were caught in Autumn 2005 and measured and pin feather samples were taken for DNA analysis. They were categorised using the sexing criteria and the results compared to sex determination based on the molecular analysis.

Discriminant Function Analysis

The morphological data were subjected to multivariate analysis by discriminant function analysis using the SPSS 12.0.1 statistical software package. Discriminant function analysis has been used previously to create morphological sexing criteria for many species (Lorentsen and Rov 1994; Glahn and McCoy 1995; Zavalaga and Paredes 1997). The discriminant function analysis involved all *D. brachypterus* that were not missing data (n = 46). The unstandardised discriminant function was then validated using a cross validation technique where each bird is classified by the function derived from all data other than that bird (Rencher 2002). The assumption for discriminant function analysis that there is homogeneity of within-group variance-covariance matrices was tested using Box's M-statistic test (Rencher 2002).

In both determining criteria for sexing and the discriminant function analysis, not all measures were available for all birds. Hence, sample sizes vary in the following analyses.

Results

Sexing Criteria

The DNA results were returned stating the sex of each bird, 25 male and 21 female. Male *D. brachypterus* were heavier and had longer wings, tail and head-bill lengths than females (Fig 1, weight $t_{33} = 5.67$, $P < 0.001$, wing length $t_{46} = 3.81$, $P < 0.001$, tail length $t_{45} = 3.39$, $P = 0.001$ and head-bill length $t_{29} = 3.59$, $P = 0.001$). A bonferroni correction was used to accommodate for multiple comparisons. As wing length and tail length are subject to error resulting from wear during the year and the difficulties of repeatable accurate measurement of these morphometrics, these measures have not been included in the development of sexing criteria. Weight had the largest relative distance between the sexes means which is useful for developing criteria, although it can also be annually and diurnally variable. Combining weight with a measure of body size such as head-bill length was used to provide a correcting influence for likely variations in weight.

To determine criteria for sexing *D. brachypterus*, the pooled standard deviation (Afifi *et al.* 2004) of the morphometric measurements was used to determine upper and lower bounds for weight and head-bill length (Table 1). When weight and head-bill length were combined, of the 15 test birds 12 were consistent with DNA sexing (80%), one was not consistent (7%) and two were inconclusive (13%). Assuming my sample of *D. brachypterus* is representative and weight and head-bill length in *D. brachypterus* are normally distributed, then the probabilities of mis-identification are shown in Table 1. Normality has only been assumed due to the difficulties associated in obtaining sample sizes large enough for a representative analysis across such a threatened and cryptic species.

Discriminant function analysis

The assumption of homogeneity of within-group variance-covariance matrices was met as they were not significantly different (Box's M-statistic = 0.795, $F_{3, 248075.5} = 0.252$, $P = 0.86$). By incorporating the same characters, weight (W) and head-bill length (HB) simultaneously into a discriminant function analysis, the following unstandardised discriminant function was obtained:

$$D = -31.388 + 0.505(W) + 0.271(HB),$$

where $D>0$ is male, $D<0$ is female.

Using a cross-validation technique where each case is classified by the function derived from all other cases other than that case, 40 of 46 birds were consistent with the DNA sexing (87%). Two females and four males were misclassified.

When examining the discriminant scores, no bird with a score above 0.27 (males) or a score below -1.02 (females) was misclassified. In estimating the probability of correctly allocating sex based on this discriminant function using Afifi *et al.* (2004) posterior probability equation, for greater than 75 % confidence in correctly sexing birds, then discriminant scores between -1.099 and 1.099 should be classed as inconclusive. A 75% confidence limit was chosen to provide a high level of discrimination whilst retaining a majority of birds in the analysis. The reliability of this function now dropped, to 46 % consistent with DNA sexing and 54 % inconclusive.

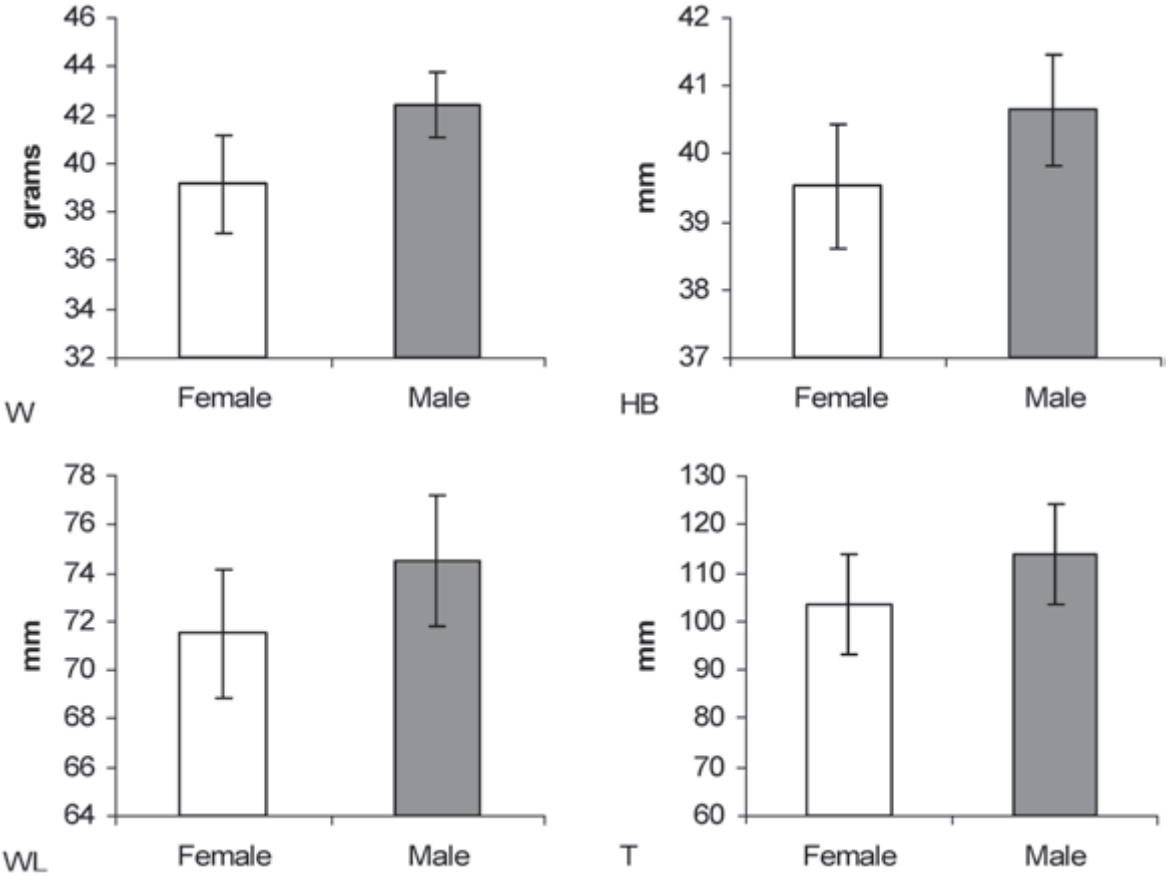

Figure 1. Morphological measurements (mean ± sd) tested for use in developing sexing criteria. W: weight, HB: head-bill length, WL: wing length and T: tail length.

Table 1. Morphological sexing criteria for the Eastern Bristlebird. Male lower boundary approximates the female average plus one pooled standard deviation (sd_p), female upper boundary approximates the male average minus one pooled standard deviation. The sex of an individual was determined by both measures indicating the same sex or one measure returning a particular sex and the other an inconclusive result. Measurements falling between the male and female bounds were classed as inconclusive. If each measure determined contradicting sexes then the sex of that bird was recorded as inconclusive. Probabilities come from a normal distribution with the corresponding means and standard deviations.

	Weight (g)	Head-bill length (mm)
Female, av + sd_p	39.2 + 1.7	39.5 + 0.9
(n)	(16)	(14)
Male rules	> 41	≥ 40.5
Probability female larger than male boundary	0.18	0.14
Male, av – sd_p	42.4 – 1.7	40.7 – 0.9
(n)	(19)	(17)
Female rules	< 41	≤ 40
Probability male smaller than female boundary	0.20	0.21

Discussion

The results suggest that both the discriminant function and the sexing criteria can be used to sex adult *D. brachypterus*. The discriminant function is easily applicable to existing data sets if there are no missing measurements, although with the inconclusive bounds it is very conservative. The sexing criteria may be more applicable in the field as no calculation is required. However, as only a simple calculation is required for the discriminant function, it is suggested that the discriminant function can be used initially and then the sexing criteria can be used to re-examine any inconclusive individuals, even in field applications.

Using this combination of techniques, the *D. brachypterus* data were re-assessed. The discriminant function left 25/46 bristlebirds inconclusively sexed, of which the sexing criteria correctly sexed 16/25, wrongly sexed 3/25 and left 6/25 inconclusive. With this combination of techniques 80 % (37/46) were consistent with DNA sexing, 7 % (3/46) were not consistent with DNA sexing and 13 % (6/46) remained inconclusive.

When re-examining inconclusive individuals from the discriminant function, the sexing criteria possibly over-estimated female numbers by incorrectly sexing juvenile males. The three wrongly sexed individuals were all

males. The inconclusive individuals included two females and four males. It is possible that the wrongly sexed and inconclusive males were juveniles, all were smaller overall than the other males and the netting of *D. brachypterus* occurred soon after breeding season. It was assumed that the majority of *D. brachypterus* caught during this study were adults. With territorial bird species, call-playback should attract nearby territory holders (Newton 1992). *D. brachypterus* display some territorial behaviour (McNamara 1946; Chapman 1999; Baker 2001; Higgins and Peter 2002) and presumably the majority of *D. brachypterus* attracted to the call playback and mist nets were adults that had reached sexual maturity and obtained home ranges. However, there was no conclusive evidence that all individuals were adults. Juveniles can be distinguished by plumage from adults at an early age, although they attain adult plumage within the first year (Higgins and Peter 2002). Another source of error may be the possible shrinkage of the museum specimens, although these were not over-represented in the inconclusive or wrongly sexed individuals.

The interaction of the two measures, weight and head-bill length, together provide the accuracy of the sexing technique. However, as weight is a seasonally variable measurement it is recommended that this sexing technique is only used outside of the breeding season, extending from August to February (Higgins and Peter 2002). Also in using measurements of adults to categorise individuals, there will inevitably be restrictions on their application to juveniles. For *D. brachypterus*, I suggest that caution be used in applying this technique during the months January to April and on individuals with a pale brown to brown iris, thought to be juveniles, rather than the red to red brown as with adults of the species (Higgins and Peter 2002). It is expected that during this period, results may under-estimate males and over-estimate females.

D. brachypterus is an endangered species and the value of this sexing technique is in its application to conservation research. Being able to determine the sex of individual birds during a translocation project, without waiting or paying for laboratory work, may provide substantial reductions in the effort and resources required to develop a viable translocated population. *D. brachypterus* is sensitive to disturbance and particularly susceptible to handling stress (Baker and Clarke 1999); the need to minimise handling is high. If a sexing technique can be based on standard morphological data usually collected during capture, handling times can be reduced. Future translocation projects will benefit from a quick field based sexing technique that minimises handling while preventing grossly skewed sex ratios of translocated birds.

Integration of this sexing technique into current research could be used to quickly and cheaply estimate sex ratios in remnant populations. However, the expansion of this analysis over a wider geographic range and on a larger sample size may be needed to increase the accuracy of the techniques. The knowledge of sex ratios in wild populations is an important component of conservation (Millar *et al.* 1997) as any attempts to develop captive populations, new populations or augment existing populations needs to understand the sex composition of the breeding system. The investigation of sex ratios has assisted in the conservation of threatened birds around the world (Double and Olsen 1997; Komdeur *et al.* 1997; Lens *et al.* 1998).

A number of birds in the Pardalotidae family in Australia are of conservation concern (Garnett and Crowley 2000). These include two other species of bristlebird, the Western Bristlebird - *Dasyornis longirostris* and the Rufous Bristlebird - *D. broadbenti*, as well as others such as the Scrubtit - King Island subspecies - *Acanthornis magnus greenianus*, Slender-billed Thornbill - Western subspecies - *Acanthiza iredalei iredalei* and Brown Thornbill – King Island subspecies - *Acanthiza pusilla archibaldi*. It has been suggested that there is no size dimorphism between the sexes in these species (Simpson and Day 1996; Higgins and Peter 2002). This sexing technique may have applications in the ongoing research projects currently investigating the conservation of these other species of bristlebird and pardalotids.

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